AD NUMBER

AD837861

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Critical Technology; JUL 1968. Other requests shall be referred to Department of the Army, Fort Detrick, Attn: Technical Release Branch/TID, Frederick, MD 21701.

AUTHORITY

BDRL, D/A ltr, 22 Oct 1971

TRANSLATION NO. 490

DATE: 1 July 68

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

UNITED STATES ARMY
CHEMICAL CORPS BIOLOGICAL LABORATORIES
Fort Detrick, Maryland

Misc Tr
490

Growth of Leptospira canicola in the presence of fatty acids.

by H. Noratz.

Translated from Zentralbl. f. Bakt. I Abt. Orig. 169:269-274 (1957) by the Technical Library, Technical Information Division.

Many attempts have been made to discover growth-promoting factors for leptospirae. To list a few examples:

According to Gsell and Wiesmann (3), addition of yeast extract or hemoglobin solution to the customary rabbit serum media had no growth-promoting effect on leptospirae. Gram and Schlipkoeter (5), on the other hand, report in 1953 that admixture of yeast, liver extract and "T-vitamin Goetsch" gave good growth.

Gram (4) tested the activity of Liebig's meat extract as substrate additive and noted death of leptospirae at a concentration of 1%, contrasted with a 2 to 3-fold increase in growth at a lower concentration of 0.5-0.6%.

In extensive investigations of the nutritional requirements of leptospirae, Green (6) compiled a minimal medium for Leptospira canicola, consisting of serum albumin, a few amino acids and vitamins.

My own tests (11) show that L. canicola grows even on protein-free media containing alcoholic extracts of rabbit serum, bovine heart or egg yolk.

The tests suggest that the ethanol-soluble growth-promoting substances could be fats or lipoids. Accordingly, tests were initiated which would examine fatty acids for their growth-promoting properties in connection with L. canicola.

Material and methods

All glassware was repeatedly rinsed with redistilled water prior to use; tubes were sterilized in hot air.

In order to have at my disposal a sufficient amount of medium, the following basic mixture was prepared:

Casein peptone "Merck" (tryptically digested)	400 mg	$CaCl_2$ (+ 30% water)		20 mg
NaCl	700 mg	KH_2PO_4		90 mg
$NaHCO_3$	10 mg	Na_2HPO_4 + 2 H_2O		480 mg
KCl	20 mg	distilled water	to	500 cc

These substances were dissolved and sterilized in the autoclave, sterilized through filter paper after cooling, bottled in lots of 50 ml and again sterilized.

The following fatty acids were tested:

butyric acid
valerianic acid
capronic acid
oenanthic acid
caprylic acid
pelargonic acid
capric acid
undecylenic acid
undecylic acid
lauric acid
myristic acid
palmitic acid
stearic acid
oleic acid
elaidic acid
ricinoleic acid

also:
succinic acid
fumaric acid
Tween 80

Fatty acids were neutralized with equinormal amounts of sodium hydroxide (p.a.); the 0.1-m solution was stored as stock supply.

A single test required:

Basic mixture			20.0 ml
Thiamine chloride (Betaxin "Bayer")			10.0 mg
Asparagin			0.1 g
Fatty acid solution (fatty acid terminal concentrations were between 10^{-2} and 10^{-8} mol.)			
Redistilled water		to	100.0 cc

A parallel series contained 1.0 g sheet gelatin. The hydrogen ion concentration was adjusted to pH 7.4 with sodium hydroxide.

The nutrient solution was placed in tubes in lots of 5 ml, closed with Kapsenberg lids (no cotton!), sterilized in the autoclave for 25 minutes at 1 atm. overpressure and stored at room temperature.

The inoculum consisted of a strain of L. canicola Zurich extant at the start of experimentation as a bovine heart solution (11). It was maintained in that medium through 22 passages.

The dosage was usually 0.5 ml per tube; in the case of poorly growing cultures we tried unsuccessfully to achieve growth with a double dose in a parallel series.

Care was taken to inoculate the same nutrient charge (same fatty acid concentration) during passage. Three tubes were inoculated simultaneously from each charge of substrate.

Results were evaluated under dry darkfield magnification (270 X).

At intervals of one week the cultures were examined and the most densely overgrown culture among 3 tubes of the last passage was used to inoculate 3 new ones. If growth was good, the series was discontinued after the 12th passage.

Each tube was examined 5 times.

Cultures which showed no growth after 3 weeks were discarded.

Results

Reproduction of the test record can be dispensed with. Results are summarized in the table. --It was not possible to grow Leptospira canicola beyond the third passage unless the medium contained gelatin.

In the presence of gelatin, only enanthic acid and valerianic acid among the short-chained fatty acids showed distinct growth promotion. Continuation failed only after the 7th passage. Of the longer fatty acids, palmitic acid, stearic acid, oleic acid and Tween 80 showed good growth. Growth was so intense in the case of palmitic acid and stearic acid that the tube showed 50-100 Leptospirae in the field one week after inoculation. 22 passages were made through nutrient containing palmitic acid. This series frequently furnished starting material for passages through other fatty acids.

The concentration of fatty acids was highly significant. Fatty acids at 10^{-2} m and 10^{-3} m inhibited growth or killed the leptospirae in the first passage. Weaker concentrations of 10^{-6} m or less no longer permitted reproduction. Most favorable were oleic acid in concentrations of 10^{-4} m and 10^{-5} m, stearic and palmitic acid at 10^{-4}.

Tween 80 gave good growth, but only in the presence of gelatin, in concentrations of 28.2 to 0.00282 mg%. The best results were achieved at higher concentrations of 28.2 and 2.82 mg%. More than 100 organisms were counted in the field.

Discussion

Unsaturated fatty acids with a chain length of 18 C-atoms have a strong growth-promoting effect in connection with many bacterial species. (Williams et al. 10, Kitay and Snell 7; for corynebacteria Pollock 9, and for clostridia Feeney et al 2 and Niemann 8). However, intensity of growth abates as the number of double bonds increases. In relatively few cases saturated fatty acids may lead to expanded growth.

In the tests described above we found good growth upon addition of oleic acid as well as palmitic acid and stearic acid. A simple unsaturated fatty acid consequently acts as effectively as saturated fatty acids. Very favorable results were noted after admixture of Tween 80.

Fatty acids with cis-configurations frequently influence the growth of bacteria more favorably than the corresponding transforms (Niemann 8). Comparison of results achieved with oleic acid and elaidic acid shows a considerably higher intensity in the case of oleic acid. Leptospiral cultures in a milieu of elaidic acid broke off in the eighth passage.

This result agrees only in part with an earlier study (12) in which L. canicola was successfully cultured up to the 12th passage in a medium containing elaidic acid.

It was not possible to maintain leptospirae beyond the third passage without gelatin additive. It was noted in the case of nutrients containing gelatin that the consistency of tubes which showed good growth in contrast to uninoculated controls had decreased after cooling to room temperature. Dubos (1) found that serum albumin is required for the cultivation of M. tuberculosis on media containing oleic acid. Dubos (1) assumed that serum albumin acts as a buffer. Large amounts of fatty acids are bound initially, which are then released continuously in small quantities. It is not clear whether the substances reabsorbed from gelatin are identical with the growth-promoting factors, or whether gelatin merely exerts a detoxifying effect, causing the growth-promoting property of fatty acids for L. canicola to come into play.

A comparison of results upon addition of oleic acid plus gelatin and Tween 80 plus gelatin to the nutrient solutions shows that Tween 80 induces better growth of L. canicola than oleic acid. Tween 80 produced good to very good growth at all utilised concentrations from 28.2 mg% to 0.00282 mg%, while oleic acid furthered multiplication only at concentrations of 10^{-5} m and 10^{-6} m. A difference between the two substances is noticeable even in non-gelatinous media. Although a fourth passage failed to materialize in either case, growth was more intense in media with Tween 80.

Niemann (8) mentions the marked growth-promoting properties of Tween 80; he explains them by the fact that in this substance oleic acid (of which Tween 80 is the polyoxyethylene-sorbitane ester) is contained within a water-soluble molecule. This is said to constitute "detoxification" of oleic acid. An interpretation of the manner in which gelatin affects the growth of L. canicola becomes even more complicated thereby.